THE INCIDENCE OF *CLOSTRIDIUM BOTULINUM* ON FRESH FROZEN FOODS

BY

ERNEST MAYNARD WEBER

A.B., University of Illinois, 1936

M.S., University of Illinois, 1938

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN BACTERIOLOGY IN THE GRADUATE SCHOOL OF THE UNIVERSITY OF ILLINOIS, 1940

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I. Introduction

Because fresh frozen foods have not been incriminated in outbreaks of food poisoning or food borne infection, an attitude probably prevails which considers such a possibility too remote for concern. The fact that no outbreaks of disease have been caused by food preserved by cold, presence of disease producing organisms on this particular product was questioned. Therefore, the purpose of this work was to point out the possible presence of *Clostridium botulinum*, the etiologic agent of botulism, on fresh frozen-pack foods purchased on the open market. In conjunction with this work several related problems were investigated and are also reported.

II. Literature Survey

A survey of the literature dealing with several aspects of the probable presence of *Clostridium botulinum* on fresh frozen foods has been presented. It was shown that the spores of this organism are probably ubiquitous and that fruits and vegetables might easily carry these spores from contaminated soils. Freezing, in general, has no great germicidal effect and it manifests no destructive power against either the spores or toxin of *Clostridium botulinum*. Some organisms, even a few pathogens, have been shown to actually multiply at freezing temperatures.

Some organisms, when grown simultaneously with *Clostridium botulinum*, will weaken or actually inhibit toxin production by the latter. Other reports indicate that this action might be influenced by the medium which is used.

III. Experimental Technique

As already stated, this problem was limited to the investigation of incidence of *Clostridium botulinum* on fresh frozen foods. These foods were purchased on the open market in several different stores and were different brands. After purchase, the sample was not allowed to thaw, but was cut into smaller sections with a sterile spatula and placed in a Stevens Electric Mixer, along with several hundred cubic centimeters of sterile distilled water. After remaining in the mixer for about ten minutes the sample was so thoroughly disintegrated that it could be drawn through a ten cubic centimeter pipette. At this point some of the

samples were given a heat treatment, others were not. Two cubic centimeter amounts were then inoculated into five different enrichment media (egg-meat, liver infusion, brain infusion, pork infusion, and beef heart infusion) and one cubic centimeter was fed to a control guinea pig. Following a period of incubation contents of enrichment tubes, which were run in triplicate, were fed to guinea pigs and a stain made of each as well as individual pH determinations. Guinea pigs which died after being fed were considered as probable positive cases, but were not called positive until feeding a subculture of the original killed a second guinea pig. Cultures, therefore, which had killed two pigs were considered as containing toxin of *Clostridium botulinum*.

IV. Results

During the course of this work, forty samples of frozen foods were purchased on the open market and analyzed. The product and number of samples are as as follows: Peas, 4; Spinach, 5; Peas and Carrots, 3; Asparagus, 7; Green beans, 9; Brussels sprouts, 3; Broccoli, 2; Cauliflower, 3; Succotash, 3; and Wax beans, 1. In the course of the forty samples, five different brands are represented.

From the forty samples ten cultures were obtained which were considered as positive for *Clostridium botulinum*. These ten represented only six different vegetables and three different brands. The product and number of positive cultures supplied are as follows: Spinach, 2; Asparagus, 2; Succotash, 2; Wax beans, 2; Peas and Carrots, 1; and Broccoli, 1.

V. Inoculated Samples

The efficiency of the enrichment culturing method was checked when *Clostridium botulinum* was easily detected in a sample of heavily inoculated green beans, using the routine method of analysis. Similar detection was made with samples of asparagus containing only ten spores per cubic centimeter of the material. Positive results were obtained from heated as well as unheated samples.

VI. The Effect of Other Organisms on the Toxin of Clostridium botulinum

Various anaerobes and several organisms isolated from frozen foods were grown concomitantly with *Clostridium botulinum*.

Different combinations of numbers of organisms in the initial inoculum were prepared and the toxicity of the cultures, after incubation, was checked. It was found that all tubes which contained *Clostridium botulinum* became toxic irrespective of the numbers of other organisms present.

Clostridium botulinum was detected by the enrichment culturing method, in samples of soil which had been inoculated fifteen years ago.

VII. Conclusions

Clostridium botulinum is present, without doubt, to a limited extent on fresh frozen foods, especially vegetables. However, if the food is handled properly and kept in the frozen state until it is used, this organism should remain dormant and cause no trouble.

VITA

The author was born April 10, 1913, at Hector, Minnesota. He attended secondary schools at Grand Junction, Colorado, and was graduated from the Grand Junction High School in 1931. During the following three years he attended classes at the Mesa State Junior College at Grand Junction. In the fall of 1934 he entered the University of Illinois and received the degree of Bachelor of Arts, with a major in Bacteriology, during the summer of 1936. The following fall he enrolled in the Graduate School of the University of Illinois and at the same time was appointed to a one-half time teaching assistantship in the Department of Bacteriology, a position which he held for the duration of graduate study. The degree of Master of Science was received February 15, 1938.

Since August, 1940, the author has been employed as Bacteriologist for the Pabst Brewing Co. of Peoria Heights, Illinois. He is an active member of Sigma Xi, a member of Phi Sigma, Gamma Alpha, and the Society of Illinois Bacteriologists.

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